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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/805,973	03/22/2004	Chengyan Zhao	038867/271254	1245

55392 7590 11/21/2007
ALSTON & BIRD LLP
BASF CORPORATION
BANK OF AMERICA PLAZA
101 SOUTH TRYON STREET, SUITE 4000
CHARLOTTE, NC 28280-4000

EXAMINER

KAPUSHOC, STEPHEN THOMAS

ART UNIT	PAPER NUMBER
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1634

MAIL DATE	DELIVERY MODE
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11/21/2007

PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary

Application No.

10/805,973

Applicant(s)

ZHAO ET AL.

Examiner

Stephen Kapushoc

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 31 August 2007.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-9, 14-24 and 29-31 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☒ Claim(s) 20-22 and 24 is/are allowed.
- 6) ☒ Claim(s) 1-9, 14-19, 23, 29-31 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

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DETAILED ACTION

Claims 1-9, 14-24 and 29-31 are pending.

Claims 10-13, 25-28, and 32-41 are cancelled.

Please note: The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

This Office Action is in reply to Applicants' correspondence of 8/31/2007.

Applicants' remarks and amendments have been fully and carefully considered but are not found to be sufficient to put the application in condition for allowance. No new grounds of rejection are presented in this Office Action. Any rejections or objections not reiterated herein have been withdrawn in light of the amendments to the claims or as discussed in this Office Action.

This Action is made **FINAL**.

Withdrawn Objection to the Specification

1. The objection to the disclosure as presented in the previous Office Action is **WITHDRAWN** in light of the amendment to the specification, which is entered.

Withdrawn Claim Objections

2. The objection to claims 1, 15 and 30 as presented in the previous Office Action is **WITHDRAWN** in light of the amendments to those claims.

Maintained Claim Rejections - 35 USC § 112 2nd ¶ - Indefiniteness

3. Claims 1-9, and 14 are unclear because while the preambles of independent claims 1, 6, and 9 recites 'method for detecting a mutant allele', there is no step in which any mutant allele is in fact detected. The claims may be made more clear if step (c) is

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amended to clearly indicate that the final process step relates to the purpose of the claim as stated in the preamble of the claim.

For example, step (c) of claims 1, 6 and 9 may include 'wherein detecting said PCR product indicates the presence of a mutant allele of a wheat *AHASL* gene'.

Response to Remarks

Applicants have indicated (Remarks p.15 of 20) that claims 1, 6, and 9 have been amended to point out more distinctly that the claimed methods involve detecting a PCR product. While step (c) of each of claims 1, 6, and 9 recites 'detecting', the aforementioned steps recite detecting a product of said PCR amplification, not a mutant allele. The Examiner maintains that the rejected claims are specifically drawn to methods for 'detecting a mutant allele', and that the claims do not clearly indicate that a mutant allele is detected. A suggestion for amendment of the claims to clearly indicate that the claimed method steps result in the stated purpose of the claimed method is set forth in the rejection.

The rejection as set forth is **MAINTAINED**.

Maintained Claim Rejections - 35 USC § 103

4. Claims 1, 8, 14, 15, 23, and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hucl et al (2003) (WO 03/014357) in view of Liu et al (1997) and Kwok et al (1990).

Hucl et al teaches the molecular basis of imidazolinone resistance in wheat plants. Regarding claim 1, the reference teaches that resistance to imidazolinone can

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be conferred by a guanine to adenine substitution in the AHASL1 gene (referred to in Hucl et al as Asl1), which results in a serine to asparagine substitution (p.7 Ins. 1-15; Figure 8 page 17/42; p.13 Ins 2-3; Figure 13). Hucl teaches the nucleic acid and deduced amino acid sequences of the AHASL1 genes of several imidazolinone resistant wheat plants (Fig 8) including the mutation responsible for herbicide resistance. The reference teaches that imidazolinone resistant mutant alleles can be detected by amplifying AHASL1 genes and comparing the amplified gene sequence to that of a known wild-type control (p.17 ln.33-p.18 ln.13; p.20 ln.26-p.21 ln.2). Relevant to step (a) of claim 1, the reference teaches the use of genomic DNA (p.18 ln.11). Relevant to step (b) of claim 1, Hucl et al teaches the portion of the AHASL1 nucleic acid sequence that is responsible for the imidazolinone resistance-mutation, including nucleotides 3-23 of SEQ ID NO: 12 of the instant application (for example see Fig 8, p17/42 of the figures, (SEQ ID NO: 15) the nucleotides encoding the amino acid sequence HVLPMIP(N/S) beginning at amino acid 620).

Regarding claim 8, Hucl teaches that there are AHAS genes on genomes A, B, and D of the *Triticum* wheat plant (p.9 Ins. 25-33), and teaches the sequence of the *Imi1* wheat gene (Fig 8; p.7 Ins.1-15), which is the AHASL1D gene on the D genome, as evidenced by Pozniak et al (2004) (p.1439 – Chromosome location of AHAS genes).

Regarding claims 15 and 23, the teachings of Hucl et al are applied to steps (a), (b), and (d) of claim 15 as they were applied to claims 1 and 8 earlier in this rejection. Additionally, Relevant to step (c) of claim 15, Hucl et al teaches the wild-type AHASL1 nucleic acid sequence that is responsible for imidazolinone sensitivity, including the

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sequence relevant to SEQ ID NO: 10 of the instant application (for example see Fig 8, p17/42 of the figures, (SEQ ID NO: 21) nucleotides that encode amino acids 621-633, HVLPMIPSGGAFKD).

Regarding claim 23, Hucl teaches that there are AHAS genes on genomes A, B, and D of the *Triticum* wheat plant (p.9 Ins. 25-33), and teaches the sequence of the *Imi1* wheat gene (Fig 8; p.7 Ins.1-15), which is the AHASL1D gene on the D genome, as evidenced by Pozniak et al (2004) (p.1439 – Chromosome location of AHAS genes).

Hucl does not teach the analysis of AHASL1 genes via allele specific PCR using oligonucleotide primers, or primers with mismatches as are required for primers directed to nucleotides 3 to 23 of SEQ ID NO: 12 wherein the 3'-end nucleotide is cytidine (step (b) of claims 1 and 15).

Liu et al teaches a method for the detection of single nucleotide polymorphisms using allele-specific primers (p.390 – Principle of Bi-PASA). Relevant to step (b) of claim 1, the reference teaches a PCR reaction comprised of genomic DNA as a template (p.397 - Methods), dNTPs, a polymerase, forward and reverse gene specific primers (referred to in the reference as primers P and Q), and a mutant-allele-specific primer (referred to in the reference as primer A) (Figure 1). The reference teaches examples in which the mutant and wild-type allele specific primers are designed to flank the polymorphic position (Fig 1; Table 1). Relevant to step (c) of claim 1, Liu et al teaches the detection of PCR products using gel electrophoresis and ethidium bromide staining (p.397 - Methods). Liu et al teaches that an allele specific primer is capable of

annealing to a region of the analyzed gene that is nested between the annealing sites of the gene-specific primers (Fig. 1).

Regarding claims 14 and 29, Liu et al teaches the detection of PCR products using gel electrophoresis and ethidium bromide staining (p.397 - Methods).

Relevant to step (c) of claim 15, Liu et al also teaches the use of wild-type allele-specific-primers for detection of wild-type alleles (Fig 1; Table 1).

Neither Hucl nor Liu teach the use of an allele specific primer with a 3'-terminal cytidine where said primer is used to detect an A nucleotide allele by specifically hybridizing to a T in the template nucleotide.

Kwok provides general teachings concerning the use of different 3'-terminal nucleotides in the amplification of template bases. Kwok et al particularly teaches (p.1001, Table III) the refractory nature of a 3'-terminal C in the amplification of a C-containing template, but the ability of a primer containing a 3'-terminal C in the amplification of a T-containing template.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the mutation detection methods of Hucl et al so as to have used allele-specific and gene-specific primers as taught by Liu et al and have used a primer with a 3'-terminal C for the specific amplification of the mutant allele. One would have been motivated to use the methods of Liu et al based on the teaching of Liu et al that a nested allele-specific amplification method provides an internal positive control (p.391, right col., Ins.6-8). One would have been motivated to use a mutant-allele specific primer with a 3'-terminal C for the detection of the mutant

allele based on the teachings of Kwok et al that such a primer will amplify a T nucleotide template but not a C nucleotide template and the teachings of Hucl et al that an herbicide resistance in wheat is based on a G-to-A (thus a template C-to-T) substitution mutation.

Response to Remarks

Applicants have traversed the rejection of claims under 35 USC 103 as obvious in light of the teachings of Hucl et al (2003) (WO 03/014357) in view of Liu et al (1997) and Kwok et al (1990). Applicants argue (Remarks p.17-18) that a mutant allele specific primer with a mismatch at the 3'-end is unexpected in view of the teachings of Newton et al and Wu et al because the references teach that a primer with a mismatched 3'-end-residue will not function as a primer. Applicants further argue that DelRio-LaFreniere et al teaches away from primers with mismatches at the 3'-end nucleotide in favor of mismatches at the penultimate and antepenultimate bases. Applicants argue that in view of the teachings of the prior art of Newton et al, Wu et al, and DelRio-LaFreniere et al one of ordinary skill in the art would not find that the teachings of Kwok et al render obvious the claimed invention requiring using a primer with a 3'-terminal C that mismatches with a T as a primer to detect the T-containing allele. Applicants conclude that the Examiner has selected in hindsight only those references that allegedly support the case of obviousness while ignoring the teachings of references that conflict with the obviousness of the claimed invention, and have invited the Examiner to particularly point out the teachings in the applied Kwok et al reference that discredit the alleged conflicting teachings of Newton et al, Wu et al, and DelRio-

LaFreniere et al. Applicants' arguments have been fully and carefully considered but are not found to be persuasive.

Initially it is noted that Applicants have mischaracterized the teachings of the references that allegedly conflict with the teachings of Kwok et al in the obviousness of the rejected claims. The claims specifically require using a primer with a 3'-terminal C, that mismatches with a T in the template, as a primer to detect a T-containing allele. The teachings of Newton et al do not include (Fig 2) any specific teaching to indicate that the required primer-template mismatch would not be functional as a PCR primer. Furthermore Newton et al clearly indicates that 'in some instances a single 3'mismatch base does allow amplification to proceed' (p.2504, Ins.24-26). Similarly the teachings of Wu et al does not offer (p.2757 – PCR) any specific teaching to indicate that the required primer-template mismatch would not be functional as a PCR primer. Wu et al the study uses only A-A and T-T mismatches, and that it is not clear that other mismatches will give equally effective discrimination (p.2758, right col., last ¶). With regard to the teachings of DelRio-LaFreniere et al, the reference provides no teachings regarding a primer with a 3'-terminal C that mismatches with a T in the template. And while Applicants allege that the reference teaches away from primers with mismatches at the 3'-end nucleotide in favor of mismatches at the penultimate and antepenultimate bases, a more accurate appraisal of the teachings of DelRio-LaFreniere et al is that mismatches at the penultimate and antepenultimate effect primer efficiency; there is no teaching away from the use of a 3'-terminal mismatch for amplification.

In contrast to the lack of specific teachings of Newton et al, Wu et al, and DelRio-LaFreniere et al, where the aforementioned references provide no specific teachings regarding the required mismatch of the claims (i.e.: a primer with a 3'-terminal C that mismatches with a T in the template), Kwok et al, as cited in the rejection, provides specific guidance with regard to the use of a primer for amplification where a 3'-terminal C in the primer mismatches with a T in the template but not a C (as required for the instant allele discrimination). Furthermore, such a primer-C:template-T mismatch was known in the prior art as summarized in Table 1 of Ayyadevara et al (2000) (as cited by Applicant as citation number 14 on the IDS of 7/30/2004; the citation is referenced in this Response to Remarks only in response to Applicants' argument concerning the pertinent knowledge of the skilled artisan, and is not required for the rejection as set forth in this Office Action). Ayyadevara et al summarizes the teachings of four different studies, including Kwok et al cited in the rejection, regarding 3'terminal primer:template mismatches, where the summary indicates that a 3'-terminal C can amplify a template T while offering specificity over a template C.

Thus the Examiner maintains that in view of the teachings of Kwok et al the artisan of ordinary skill would be motivated to perform the claimed method using a primer with a 3'termial C for the detection of the T nucleotide of the AHASL mutant allele.

The rejection as set forth is **MAINTAINED**.

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5. Claims 2, 4, 5, 16, 18, 19, and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hucl et al (2003) (WO 03/014357) in view of Liu et al (1997) and Kwok et al(1990), and further in view of Stanton (2002) US Patent 6,475,736.

The teachings of Hucl et al in view of Liu et al and Kwok et al are applied to claims 2, 4, 5, 16, 18, 19, and 30 as they were previously applied to claims 1, 8, 14, 15, 23, and 29 earlier in this office action.

Hucl et al in view of Liu et al and Kwok et al teaches a method for the detection of mutant AHASL alleles that confer tolerance to imidazolinone on a wheat plant, including the use of a mutant-allele-specific primer with a 3'-terminal cytidine.

Hucl et al in view of Liu et al and Kwok et al does not teach a pre-amplification step using primers that amplify a product that contains nested annealing sites for the gene-specific primers used to detect specific mutations.

Stanton teaches methods for the analysis of DNA using amplification of polymorphic sites. Relevant to claims 2, 16, and 30 step (b), Stanton teaches that the PCR amplification step of a genotyping procedure can be modified to increase sensitivity by using nested PCR (two rounds of PCR, first with an outside set of primers, then with an inside set) (col.34 Ins.35-39).

Regarding claims 4 and 18, Hucl teaches that there are AHAS genes on genomes A, B, and D of the *Triticum* wheat plant (p.9 Ins. 25-33), and provides an alignment of the three different imidazolinone resistance genes (Fig. 12). The sequences taught by Hucl et al include primer binding sites for which it would be a

necessary property that oligonucleotide primers directed to those regions would anneal to AHASL1A, AHASL1B, and AHASL1D.

Regarding claims 5 and 19, which depend from claims 2 and 16 respectively, Hucl teaches the AHASL1 sequence that includes the sequence relevant to SEQ ID NO: 1 of the instant application (for example see Fig 12, p39/42 of the figures, nucleotides 901-920).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the mutation detection methods of Hucl et al in view of Liu et al and Kwok et al so as to have incorporated a pre-amplification step as taught by Stanton. One would have been motivated to do so based on the teachings of Stanton that a pre-amplification step can increase the sensitivity of the methods (col 34 lns.36-37). One would have had a reasonable expectation of success because Stanton teaches the pre-amplification step in association with PCR based methods, and the allele detection methods Liu et al are PCR based. Further regarding claims 5 and 19, it would be obvious to use primers comprising the claimed sequence (SEQ ID NO: 1) given the alignment of the three imidazolinone resistance genes (Fig. 12 of Hucl et al) and the consensus sequence that indicates this region is conserved among the three genes, as use of such a primer would allow for subsequent analysis of any of the three AHASL1 genes from any of the three wheat genomes.

Response to Remarks

Applicants have traversed the rejection of claims under 35 USC 103 as obvious in light of the teachings of Hucl et al (2003) (WO 03/014357) in view of Liu et al (1997)

and Kwok et al (1990) and in further view of Stanton et al. The traversal is on the grounds that one of ordinary skill would not find that the teachings of Kwok et al render obvious the claimed invention because of other allegedly conflicting references. This argument has been addressed in the previous Response to Remarks.

The rejection as set forth is **MAINTAINED**.

6. Claims 3, 17, and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hucl et al (2003) (WO 03/014357) in view of Liu et al (1997), Kwok et al (1990) and Stanton (2002) US Patent 6,475,736 and further in view of Werle et al (1994).

The teachings of Hucl et al in view of Liu et al, Kwok et al and Stanton are applied to claims 3, 17, and 31 as they were previously applied to claims 2, 4, 5, 16, 18, 19, and 30 earlier in this office action.

Hucl et al in view of Liu et al, Kwok et al and Stanton teaches a method for the detection of mutant AHASL alleles that confer tolerance to imidazolinone on a wheat plant. The method utilizes a pre-amplification step, followed by the use of allele-specific primers with intentional mismatches for amplification, and uses mutant and wild-type specific primers.

Hucl et al in view of Liu et al, Kwok et al and Stanton does not teach the use of an exonuclease following the pre-amplification step.

Werle et al teaches the use of exonuclease I to degrade excess primers and nucleotides from PCR products prior to analysis by sequencing. Relevant to claims 3, 17, and 31, Werle et al teaches pre-amplification of a PCR product from genomic DNA,

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followed by treatment with exonuclease, then analysis of the exonuclease treated PCR product using the same conditions as for PCR of genomic DNA (p.4354, Ins.20-36).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the mutation detection methods of Hucl et al in view of Liu et al, Kwok et al and Stanton so as to have incorporated an exonuclease digestion step as taught by Werle et al. One would have been motivated to do so based on the teachings of Werle et al that exonuclease digestion removes factors that interfere with analyses that utilize PCR based methods (p.4354 In.13-16; p.4354 In.34-37), and that the exonuclease method is simple to use with minimum sample handling, risk of cross-contamination and amount of DNA template required, and the method is reliable, convenient, and cost effective (p.4355 Ins.1-6). One would have had a reasonable expectation of success because Werle asserts that the method has broad applicability in mutational analysis by a PCR based method (p.4355 Ins.6-8), and the allele-specific amplification method Liu et al is a PCR based method.

Response to Remarks

Applicants have traversed the rejection of claims under 35 USC 103 as obvious in light of the teachings of Hucl et al (2003) (WO 03/014357) in view of Liu et al (1997), Kwok et al (1990) and Stanton et al, in further view of Werle. The traversal is on the grounds that one of ordinary skill would not find that the teachings of Kwok et al render obvious the claimed invention because of other allegedly conflicting references. This argument has been addressed in the previous Response to Remarks.

The rejection as set forth is **MAINTAINED**.

Conclusion and Claim Objections

7. No rejections under 35 USC 102 or 35 USC 103 are made against claims 6, 7, 9, 20-22, or 24. The novelty of claims requiring SEQ ID NO: 2 (i.e. claims 6 and 20) and SEQ ID NO: 7 (i.e. claims 9 and 24) has been addressed previously in the Office Actions of 03/16/2006 and 09/11/2006.

Regarding claims that require SEQ ID NO: 3 (claims 7 and 21) or SEQ ID NO: 4 (claim 22), it is noted that these allele specific primers contain an intentional mismatch at the -4 position (i.e. each primer contains a G three nucleotides from the 3'-terminal nucleotide, where the G is in a G:G mismatch with the template DNA). There is no motivation in the prior art to specifically create allele-specific oligonucleotide primers in which the -4 position has a G:G mismatch with the template DNA.

Claims 20, 21, 22 and 24 are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen Kapushoc whose telephone number is 571-272-3312. The examiner can normally be reached on Monday through Friday, from 8am until 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached at 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Stephen Kapushoc
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RAM R. SHUKLA, PH.D.
SUPERVISORY PATENT EXAMINER